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PHARMACEUTICAL PREPARATIONS AND MEDICAMENTS FOR THE PREVENTION AND TREATMENT OF ENDOTHELIAL DYSFUNCTION

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(57) Claim

1. Method for the prevention, treatment and elimination of endothelial dysfunctions and of disorders associated with these dysfunctions and/or caused thereby, selected from

a) endothelial damage due to hypercholesterolaemia,

b) endothelial damage due to hypoxia,

c) endothelial damage due to mechanical and chemical factors, in particular during and after pharmacological and mechanical reopening of stenosed vessels, for example after percutaneous transluminal angiography [sic] (PTA) and percutaneous transluminal coronary angiography [sic] (PTCA),

d) endothelial damage in the postinfarct phase (endothelial dysfunction associated with reperfusion),

e) endothelial-mediated reocclusion after bypass surgery,

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f) disturbances of blood flow in peripheral arteries due to atherosclerotic changes in vessel walls, and atherosclerosis in general,

g) hypertension, including pulmonary and portal hypertension,

i) diabetic micro- and macroangiopathy and

j) heart failure,

by use of compounds which release and/or transfer nitrogen monoxide, with the homocysteinaemia acquired or induced by a genetically related enzyme defect, and the disorders caused thereby, being excepted in each case.

2. Method according to Claim 1, characterized in that the compounds used are chosen from:

a) organic nitrates, in particular glycerol trinitrate (GTN), pentaerythrityl tetranitrate (PETN), isosorbide 5-mononitrate (ISMN), isosorbide dinitrate (ISDN), mannitol hexanitrate, inositol hexanitrate, propatyl nitrate, trolnitrate, nicorandil or SPM 3672, and their pharmacologically suitable derivatives,

b) organic nitrites such as isoamyl nitrite,

c) thionitrites,

d) thionitrates,

e) nitrosothiols such as S-nitroso-N-acetyl-D,L-penicillamine (SNAP),

f) nitrosoproteins, nitrogen monoxide-liberating furoxan derivatives,

g) nitrogen monoxide-liberating sydnone imine derivatives, especially mesocarb, molsidomine or their pharmacologically active metabolites,

h) nitrosyl complex compounds such as iron-nitrosyl compounds, in particular sodium nitroprusside, and

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i) nitrogen monoxide (NO) itself.

3. Method according to Claim 1 or 2, characterized in that the compounds are used in combination with other active substances used for the treatment of cardiovascular disorders, in particular with those from the indication groups of ACE inhibitors, antiatherosclerotics, antihypertensives, beta blockers, cholesterol-lowering agents, diuretics, calcium antagonists, coronary dilators, lipid-lowering agents, peripheral vasodilators or platelet aggregation inhibitors.

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(54) Title: PHARMACEUTICAL PREPARATIONS AND MEDICAMENTS FOR THE PREVENTION AND TREATMENT OF ENDOTHELIAL DYSFUNCTION

(54) Bezeichnung: PHARMAZEUTISCHE ZUBEREITUNGEN UND ARZNEISTOFFE ZUR PRÄVENTION UND BEHANDLUNG ENDOTHELIALER DYSFUNKTION

(57) Abstract

The present invention describes the use of compounds which release or transfer nitrogen monoxide, of endogenous nitrogen monoxide formation stimulators, and of guanylate cyclase stimulators for preventing, treating and eliminating endothelial dysfunctions and diseases associated with or caused by said dysfunctions. The invention further describes the use of said compounds for preparing pharmaceutical products for said areas of application.

(57) Zusammenfassung

Vorliegende Erfindung beschreibt die Verwendung von stickstoffmonoxidfreisetzenden- oder übertragenden Verbindungen, von Stimulatoren der endogenen Stickstoffmonoxidbildung sowie von Stimulatoren der Guanylatzyklase zur Prävention, Behandlung und Beseitigung endothelialer Dysfunktionen und von mit diesen Dysfunktionen einhergehenden oder durch sie hervorgerufenen Erkrankungen sowie die Verwendung besagter Verbindungen zur Herstellung von pharmazeutischen Erzeugnissen für die benannten Anwendungsgebiete.

Pharmaceutical preparations and medicinal substances for the prevention and treatment of endothelial dysfunctions

Description

Area of application of the invention

The invention presented herein relates to the use of compounds which release and/or transfer nitrogen monoxide, of stimulators of endogenous nitrogen monoxide formation and of stimulators of guanylate cyclase for the prevention, treatment and elimination of endothelial dysfunctions and of disorders caused by endothelial dysfunctions or associated therewith. The invention simultaneously makes it possible to provide pharmaceutical preparations for said indications.

Known technical background

Organic esters of nitric acid such as glycerol trinitrate (GTN) (Murrel, Lancet: 80, 113, 151 (1879), pentaerythrityl tetranitrate (PETN) (Risemann et al., Circulation, Vol. XVII, 22 (1958), US-A 2 370 437), isosorbide 5-mononitrate (ISMN) (DE-A 22 21 080, DE-A 27 51 934, DE-A 30 28 873, DE-C 29 03 927, DE-A 31 02 947, DE-A 31 24 410, EP 45 076, EP 57 847, EP 59 664, EP 64 194, EP 67 964, EP 143 507, US-A 3 886 186, US-A 4 065 499, US-A 4 417 065, US-A 4 431 829), isosorbide dinitrate (ISDN) (L. Goldberg, Acta Physiolog. Scand. 15, 173 (1948)), propatyl nitrate (Médard, Mem. Poudres 35: 113 (1953)), trol nitrate (FR 984 523) or

nicorandil (US-A 4 200 640) and similar compounds are vasodilators, some of which have been very widely used for decades primarily for the indication of angina pectoris or ischaemic heart disease (IHD) (Nitrangin®, Pentalong®, Monolong®, Isoket®, Elantan® etc.). Comparable and improved pharmacological activity on use in the abovementioned indication areas is shown by organic nitrates of a more recent type such as, for example, SPM 3672 (N-[3-nitratopivaloyl]-L-cysteine ethyl ester) (US-A 5 284 872) and its derivatives. The use of organic nitrites such as isoamyl nitrite as coronary dilators has also been known for a long time (Brunton, Lancet 97 (1867)). Other compounds which release or transfer nitrogen monoxide, such as, for example, thionitrites, thionitrates, S-nitrosothiols or nitrosoproteins (Harrison et al., Circulation 87: 1461-1467 (1993)), and substituted furoxans (1,2,5-oxadiazole 2-oxides, furazan N-oxides) (Feelisch et al., Biochem. Pharmacol. 44: 1149-1157 (1992) or substituted sydnone imines, especially molsidomine (DE-B 16 95 897, DE-B 25 32 124, DD 244 980) have likewise been described as potent coronary dilators. All these substances are able, themselves or in the form of their pharmacologically active metabolites, for example the molsidomine metabolites "SIN 1" and "SIN-1A" (Noack, Nitroglycerin VII, Walter de Gruyter & Co., Berlin 1991, 23-28) and their derivatives and structural analogues (Noack and Feelisch, Molecular mechanism of nitrovascular bioactivation, in "Endothelial Mechanisms of Vasomotor Control" (Ed. Drexler et al.), pp. 37-50,

Steinkopff Verlag, Darmstadt, F.R.G. (1991)), to liberate or transfer nitrogen monoxide in vivo.

The pharmaceutical processing of the organic nitrates and nitrites and of other compounds which liberate or transfer nitrogen monoxide to give pharmaceutical preparations for the treatment of angina pectoris and of ischaemic heart disease are [sic] generally known. It takes place in accordance with the operating procedures and rules which are generally familiar to the person skilled in pharmacy, with the selection of the technologies to be used and the pharmaceutical ancillary substances employed depending primarily on the active substance to be processed. Of particular importance in this connection are questions concerning its physicochemical properties, the chosen administration form, the required duration of action and the avoidance of medicinal substance/ancillary substance incompatibilities. Particularly described for medicinal products for the indication of angina pectoris or ischaemic heart disease is oral, parenteral, sublingual or transdermal administration in the form of tablets, coated tablets, capsules, solutions, sprays or plasters (DD 293 492, DE-D 26 23 800, DE-A 33 25 652, DE-A 33 28 094, DE-C 40 07 705, DE-A 40 38 203, JP Application 59/10513 (1982)).

Besides the uses, which have been known for many years, are substances with nitrosating activity, their use for the treatment and prevention of disorders which are caused by pathologically elevated concentrations of

sulphur-containing amino acids in body fluids has been described. These pathological states, [lacuna] are brought about by inborn or acquired defects in the metabolism of these amino acids and which are characterized by elevated concentrations in the blood and urine of said amino acids (homocystinuria), are collectively referred to as homocysteinaemia (WO 92/18002).

The antiischaemic activity of the organic nitrates and the other abovementioned classes of substances is explained by haemodynamic effects, in particular a heart-relieving effect, which leads to a saving in the oxygen consumption of the heart and corrects the imbalance between O_2 supply and demand which is present in IHD. The cause is a preferred dilatation of the venous capacity vessels (venous pooling) or reduction in the preload and a direct coronary-dilating effect, especially in the region of coronary stenoses. This possibly beneficially affects precisely post-stenotic hypoperfusion (positive steal effect) because the organic nitrates evidently have more potent effects in atherosclerotic vascular areas than in healthy vascular sections (Kojda et al., Endothelium 1 (Suppl.) Abstr. 299 p. 76 (1993)), especially in the region of coronary stenoses. This purely haemodynamic effect is mediated by free-radical nitrogen monoxide NO•, which is released uniformly from all nitro vasodilators despite the great differences between the compounds in chemical structure. The bioactivation pathways which eventually lead to provision of NO· at the site, that is to say in the

endothelial cell and smooth muscle cell, in the vessel, are, however, very different (Noack and Feelisch, Molecular mechanism of nitrovascular bioactivation, in "Endothelial Mechanisms of Vasomotor Control" (Ed. Drexler et al.) pp. 37-50, Steinkopf Verlag, Darmstadt, F.R.G. (1991)). It has been possible to explain this definitively in recent years by direct NO· measurement by various techniques (methods Noack et al., Neuroprotocols 1: 133-139 (1992)). NO has a vasodilating effect by activating soluble guanylate cyclase. This stimulates the formation of cGMP from GTP. cGMP in turn leads to various phosphorylation reactions (for example on protein kinases), which promote intracellular Ca storage (Karezcwski et al., Z. Kardiol, 79 (Suppl. 1): 212 (1990)). The reduction in the intracellular free Ca^{2+} level then results in the relaxation. It has been known since 1987 that endothelium-derived relaxing factor (EDRF) is identical to NO or an NO-containing substance (Palmer et al., Nature, 327: 524-526 (1987); Ignarro et al., Proc. Natl. Acad. Sci. 84: 9265-9269 (1987)) and has important significance for local perfusion.

Endothelial cells form a continuous monolayer on the inner wall of the blood vessels. In the human adult this results in a total surface area of about 800 m^2 with an intrinsic weight of 1.5 to 2 kg, which corresponds to that of the human liver. The functions carried out by the endothelial cells are, according to current views, of two types: one mechanical and one functional. On the one hand, they carry out a type of barrier function intended

to prevent the penetration of constituents of blood, such as, for example, low density lipoproteins (LDL), into the vessel wall adjacent to the lumen (intima). On the other hand, they have an endocrine function. Various stimuli result in increased synthesis of bioactive substances such as EDRF/NO and prostaglandin I_2 (PGI_2), with which the function of circulating cells (Pohl and Busse, Eur. Heart J:11 (Suppl. B) 35-42 (1990)), the regional haemodynamics (Furchgott, Circ. Res. 53: 557-573, (1983)) and the structural composition of the vessel wall (Di Corleto, Exp. Cell Res. 153: 167-172 (1984)) are fundamentally influenced. This also easily explains why on damage to the endothelium, with whatever cause (endothelial damage due to hypercholesterolaemia (T.J. Verbeuren et al., Circ. Res. 58: 552-564 (1986). Endothelial damage in the postinfarct phase (M.R. Sigreid et al., Circ. 86 (Suppl. I): 21 (1992)), results in a pathological effect on endothelial function which may have various consequences. These include regional vasoconstriction or vasospasm and metaplastic or growth processes in the vessel wall, which are regarded as initial processes of atherogenesis.

Endothelial dysfunction is generally characterized by an impairment or loss of endothelium-mediated physiological vasodilatation. There is observed to be a simultaneous reduction or abolition of the NO-mediated vasorelaxation, of the NO-mediated vessel protection and of the growth processes, suppressed by NO, in the intima and media. Endothelial dysfunction is furthermore charac-

terized by proliferative processes in the vessel wall as a consequence of increased mitogenesis, increased endothelial adhesion and migration of leucocytes and macrophages, and increased oxidation of low density lipoproteins (LDL), which are damaging to the endothelium. It is frequently observed in pathophysiological states within the framework of atherosclerosis, hypertension, hypercholesterolaemia, diabetes mellitus and heart failure (Creager et al., J. Clin. Invest. 86, 228-234 (1990); Linder et al., Circulation 81: 1762-1769 (1990); Zeiher et al., Circulation 83: 391-401 (1991)). Likewise, hypoxia and low shear forces are factors inducing endothelial dysfunction. It leads, inter alia, to vasoactive substances such as acetylcholine or serotonin, which normally cause vasorelaxation, bringing about vasoconstriction because of their direct vasoconstricting effects on smooth muscles of the vessels, which have adverse affects on the clinical picture (Golino et al., N. Engl. J. Med. 324; 641-648 (1991)). Thus, in cases of endothelial dysfunction, physiological vasomotor regulation is not only disturbed but, on the contrary, inverted. These changes are even more pronounced when there is atherosclerotic transformation of the inner wall of the vessel (Ludmer et al., N.Engl. J. Med. 315; 1046-1051 (1986)).

The endothelium not only contributes with its autocrine and paracrine activity to maintaining the health of the blood vessel wall but also influences the

effect of exogenous NO liberators such as PETN or GTN by itself forming EDRF/NO. If the endothelium is removed, for example mechanically, from the arterial wall (during invasive catether [sic] diagnosis or extracorporeally on isolated vessel segments) or if endothelial NO formation is suppressed by specific inhibitors, there is potentiation of the vasodilating effect of nitro vasodilators such as GTN or PETN (Russe et al., Cardiovasc. Pharmacol. 14 (Suppl. 11); S81-85 (1989); Kojda et al., J. Vasc. Res. 29; 151 (1992A). Pharmacological inhibition of endothelial NO synthesis leads to the same effect on coronary veins (Kojda et al., Naunyn-Schmiedeberg Arch. Pharmacol. 346; R35 (1992B)). It is known that the effect of calcium antagonists, especially those of the 1,4-dihydropyridine type (DHPs), is diminished after removal of the endothelium (Kojda et al., Bas. Res. Cardiol. 86; 254-256 (1991)). Further investigations have shown that these substances are probably stimulators of endothelial NO formation and release (Gunther et al., Basic Res. Cardiol. 87; 452-460 (1992)). Likewise kinins such as bradykinin display their biological activity via increased endothelial formation and release of EDRF/NO (V.A. Briner et al., Am. J. Physiol. 264; F322-F327 (1993); Kelm et al., Biochem. Biophys. Res. Commun. 154; 236-244 (1988)).

Description of the invention

Endothelial dysfunctions are now regarded as inducers of common and pathophysiologically significant

cardiovascular disorders such as atherosclerosis. The prevention, treatment and elimination of these dysfunctions and of disorders associated therewith or caused thereby are therefore important therapeutic necessities.

It has now been found that the use of compounds which release and/or transfer nitrogen monoxide, of stimulators of endogenous nitrogen monoxide formation and of stimulators of guanylate cyclase, in particular of stimulators of soluble guanylate cyclase, is suitable for the prevention, treatment and elimination of endothelial dysfunctions and of disorders associated with these dysfunctions and/or caused by them. These endothelial dysfunctions and disorders are, in particular, endothelial damage due to hypercholesterolaemia, endothelial damage due to hypoxia, endothelial damage due to mechanical and chemical factors, in particular during and after pharmacological and mechanical reopening of stenosed vessels, for example after percutaneous transluminal angiography [sic] (PTA) and percutaneous transluminal coronary angiography [sic] (PTCA), endothelial damage in the postinfarct phase (endothelial dysfunction associated with reperfusion), endothelium-mediated reocclusion after bypass surgery, disturbances of blood flow in peripheral arteries, and cardiovascular disorders such as atherosclerosis, hypertension, including pulmonary and portal hypertension, hypertensive heart disease, diabetic micro- and macroangiopathy, coronary heart disease, heart failure or other disorders

causally derived from endothelial dysfunctions.

Compounds which release and/or transfer nitrogen monoxide, stimulators of endogenous nitrogen monoxide formation and stimulators of guanylate cyclase for the purpose of this invention are, inter alia, compounds which act directly or indirectly on guanylate cyclase, with indirect stimulators of guanylate cyclase being regarded as compounds which are able to release stimulators of guanylate cyclase or otherwise increase the enzymically active concentration thereof and/or act as antagonists towards inhibitors of guanylate cyclase, or otherwise reduce the enzymically active concentration thereof. Indirect stimulators of guanylate cyclase which are used are, inter alia, compounds suitable for increasing endogenous NO formation or release, such as calcium antagonists, in particular those of the 1,4-dihydropyridine type, for example nifedipine, felodipine, nimodipine, amlodipine and others. It is likewise suitable to use compounds able to increase the endothelial kinin content. These are, in particular, stimulators of kinin receptors such as kinins or substances with analogous activity, stimulators of endothelial kinin formation, and inhibitors of kinin degradation, in particular inhibitors of angiotensin converting enzyme (ACE inhibitors) such as captopril, enalapril, moexipril, ramipril and related active substances. The use of compounds which display their effect generally by release and/or transfer of endogenous or

exogenous nitrogen monoxide is the particularly preferred embodiment of the present invention. Classes of substances and compounds which are specifically suitable in this connection are organic nitrates, especially glycerol trinitrate, pentaerythrityl tetranitrate, isosorbide 5-mononitrate, isosorbide dinitrate, mannitol hexanitrate, inositol hexanitrate, propatyl nitrate, trol nitrate, nicorandil, newer nitrates such as SPM 3672, and their pharmacologically suitable derivatives, organic nitrites such as isoamyl nitrite, thionitrites, thionitrates, S-nitrosothiols such as S-nitroso-N-acetyl-D,L-penicillamine, nitrosoproteins, furoxan derivatives which liberate nitrogen monoxide, sydnone imine derivatives which liberate nitrogen monoxide, in particular molsidomine, mesocarb and analogues thereof, nitrosyl complex compounds, in particular iron-nitrosyl compounds such as sodium nitroprusside, and nitrogen monoxide itself. Since the release and/or transfer of nitrogen monoxide in these cases often takes place in vivo via pharmacologically active metabolites, the latter are in principle just as suitable for use for the purpose of the present invention. It is also possible to use pharmacologically suitable derivatives of all the abovementioned compounds. Possible variants are, in particular, conventional addition compounds, salts or compounds which can be cleaved by enzymes or hydrolysis, such as esters, amides and the like.

The selection of the particular active substance

is based on general pharmacological principles and the therapeutic requirements, which are familiar to the skilled person. Besides the required pharmacological effect, account must furthermore be taken of the state of health, the state of the disease, the physical condition, the known effects and side effects, contraindications, the frequency of treatment, the duration of use, pharmaceutical interactions and parallel uses of pharmaceuticals.

Administration takes place in therapeutic doses in each case which are based on those on which the particular active substances are already used for said indications. The total daily dose may be up to 500 mg, depending on the active substance. In general, daily doses of up to 350 mg will be sufficient. Administration and the dosage interval should be chosen so that therapeutic plasma levels which are as constant as possible are set up. The compounds employed according to the invention can be used themselves or as part of a pharmaceutical preparation, as single active substance or in combination with one another or combined with known cardiovascular therapeutics, for example ACE inhibitors, antiatherosclerotics, antihypertensives, beta blockers, cholesterol-lowering agents, diuretics, calcium antagonists, coronary dilators, lipid-lowering agents, peripheral vasodilators, platelet aggregation inhibitors or other substances likewise employed as cardiovascular therapeutic agents.

Pharmaceutical preparations are produced in this connection in accordance with the operating procedures and rules which are generally familiar to the person skilled in pharmacy, with the selection of the technologies to be used and the pharmaceutical ancillary substances employed being based primarily on the active substance to be processed. Of particular importance in this connection are questions concerning its physicochemical properties, the chosen administration form, the required duration of action, the site of action and the avoidance of medicinal substance/ancillary substance incompatibilities. It is the responsibility of the skilled person to select, on the basis of known substance and process parameters, in a way which is trivial per se, the medicinal form, ancillary substances and manufacturing technology. The relevant medicinal form should in this case be designed so that, to achieve constant therapeutic plasma levels, it contains the particular active substance in an amount which makes it possible to divide the daily dose into 1 to 2 single doses in the case of release-controlled systems and into up to 10 single doses in the case of other medicinal forms. Likewise suitable is continuous administration by continuous infusion.

It is possible according to the invention for said compounds to be administered in particular orally, intravenously, parenterally, sublingually or transdermally. The particular medicinal preparation is preferably produced in liquid or solid form. Suitable for

this purpose are solutions, in particular for the preparation of drops, injections or aerosol sprays, furthermore suspensions, emulsions, syrups, tablets, film-coated tablets, sugar-coated tablets, capsules, pellets, powders, pastilles, implants, suppositories, creams, gels, ointments, plasters or other transdermal systems.

The pharmaceutical preparations contain customary organic or inorganic excipients and ancillary substances which can be employed in pharmaceutical technology and which are themselves chemically inert towards the particular active substances. Suitable for this purpose are, without restriction thereto, water, salt solutions, alcohols, vegetable oils, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, highly disperse silica, paraffin, fatty acid mono- and diglycerides, cellulose derivatives, polyvinylpyrrolidone and the like. The preparation can be sterilized and, if necessary, be mixed with ancillary substances such as bulking agents, binders, glidants, mould release agents, lubricants, disintegrants, humectants, adsorbents or antidisintegrants, preservatives, stabilizers, emulsifiers, solubilizers, salts to influence the osmotic pressure, buffer solutions, colorants, perfumes, flavourings or sweeteners. The person skilled in pharmacy will make a suitable choice based on known substance parameters to avoid medicinal substance/ancillary substance incompatibilities.

The presented invention reveals a novel therapeutic option for counteracting pathological situations which, like hypoxia, high serum cholesterol levels, elevated blood pressure, diabetes, post-stenotic reperfusion, for example in the case of a myocardial infarct and mechan. and chem. factors, inter alia, promote endothelial dysfunction, or entirely preventing the development of endothelial dysfunction. The therapeutic use of suitable compounds, irrespective of the pharmaceutical preparation, therefore permits, for the first time, as stated, the prevention and up to date treatment of cardiovascular disorders of this aetiology such as, for example, atherosclerosis and the sequelae resulting therefrom. These include coronary heart disease, vascular stenoses and disturbances of blood flow in the peripheral arteries, micro- and macroangiopathies in the framework of diabetes mellitus etc. Surprisingly, the compounds characterized above show an independent endothelium-protecting effect which is independent of the properties previously known, in particular the purely haemodynamic and antischaemic properties, of, for example, the organic nitrates or their efficacy for homocysteinaemia. Their use is therefore able to stop these pathological processes or even reverse them as long as they are not yet irreversible. The component of action is therefore unexpected and of a novel type, and has not hitherto been described, nor was it to be expected in this form.

The following examples are intended to explain the invention in detail in respect of its essence and of its implementation without, however, restricting its scope.

Examples of implementation

Example 1

Experiments on a pharmacological in vivo model (New Zealand rabbit)

Cholesterol feeding in an animal experiment is suitable for generating, within weeks or months, an endothelial dysfunction which permits the effects of drugs to be investigated and quantified (Jayakody et al., Can. J. Physiol, Pharmacol. 63: 1206-1209 (1985); Verbeuren et al., Circ. Res. 58: 552-564 (1986); Freiman et al., Circ. Res. 58: 783-789 (1986)).

Groups each of 9 female New Zealand rabbits were fed with a standard diet or a cholesterol-supplemented (0.75%) diet (40 g/kg/day) over a period of 15 weeks. The cholesterol feeding led to an increase in the plasma levels from 69.8 ± 10.4 to 907.1 ± 85.5 mg/dl and induced atherosclerotic lesions in the region of the aorta, which were quantified after staining with Sudan IV by means of a computer-assisted laser scanning technique. The aortic changes comprised an area of 73.3 ± 1.9% on the aortic arch, one of 46.3 ± 2.5% on the thoracic aorta and 49.6 ± 3.5% in the region of the abdominal aorta (Fig. 2, control).

Example 2

The vessels with atherosclerotic damage showed an unchanged contractility to phenylephrine, but the endothelium-mediated vasorelaxation after administration of 1 μM acetylcholine was altered in its function by comparison with the controls (standard diet) in a way which can best be described as endothelial dysfunction. The segments of the thoracic aorta from the animals fed with cholesterol (+) show a significantly lower sensitivity to acetylcholine than the aortic segments from the control animals (Fig. 1). The degree of the measured endothelial dysfunction correlated directly with the particular severity of the atherosclerotic lesions ($r = 0.67$, $p < 0.0001$) (Fig. 3). These data prove that endothelial dysfunction developed after feeding with cholesterol.

Example 3

Two other groups each of nine white New Zealand rabbits received in addition pentaerythrityl tetranitrate (PETN) (6 mg/kg/day), with the drug being incorporated into the pelleted feed. There was a significant reduction in the atherosclerotic lesions in the animals treated concurrently with PETN. The extent of these lesions was determined as in Example I [sic]. There was a significantly reduced proportion of atherosclerotic damage in all parts of the aorta (aortic arch: 58.6 ± 2.1%, thoracic aorta 34.7 ± 2.0% and abdominal aorta 39.3 ± 3.1%) (Fig. 2).

Example 4

Likewise after PETN feeding there was no longer observed to be a significant difference in the maximum (1 μM acetylcholine) endothelium-mediated relaxation compared with the animals not fed with cholesterol (endothelial dysfunction), so that these results indicate an unambiguous protective effect of the nitro vasodilator PETN, because the latter prevented endothelial dysfunction within the meaning of the invention presented here (Figs. 3 and 4; Table 1).

Comparison between Figure 3 and 4 shows that PETN can reduce the extent of atherosclerotic lesions and improve endothelial function. The fact that the correlation coefficient in the PETN group is worse indicates that PETN likewise leads to a dissociation of the close relation between atherosclerotic lesions and endothelial functions.

Table 1 shows the effect of PETN (6 mg/kg/day) on the development of endothelial dysfunction in the thoracic aorta of white New Zealand rabbits which was induced after feeding with a cholesterol diet (0.75%, 15 weeks). The strength of the effect of the endothelium-dependent vasodilator acetylcholine is expressed as the concentration (in -logM; pD_2 value) which, on cumulative administration, antagonized half the effect of the vasoconstrictor phenylephrine (this value increases with the strength of the effect of acetylcholine). The maximum dilating effect is expressed as the percentage of the effect of the vasoconstrictor phenylephrine which was

antagonized at the maximally effective concentration of acetylcholine (1 μM). The endothelial dysfunction induced by cholesterol feeding on its own (control) is evident from the significantly reduced strength of the effect and the maximum effect of acetylcholine (*, $p < 0.05$). The differences are no longer detectable on concurrent feeding with PETN. In addition, PETN significantly (#, $p < 0.05$) improves the strength of the effect of acetylcholine and thus endothelial function after cholesterol feeding, whereas there is a significant deterioration in endothelial function after standard feeding. Overall, this shows the protective effect of PETN on endothelial function in experimentally induced atherosclerosis. The absorption and rise in the level of PETN in the plasma can also be demonstrated 24 h after the last feeding of the animals by means of the measured concentrations of the metabolite pentaerythrityl mononitrate (PEMN) in the plasma (Fig. 5).

Table 1:

	Control		PETN	
	Standard	Cholesterol	Standard	Cholesterol
Strength of the effect of acetylcholine [pD_2 values]	6.91 ± 0.02	6.12 ± 0.05*	6.62 ± 0.06#	6.47 ± 0.13#
Max. dilating effect of acetylcholine [%]	81.8 ± 1.2	60.7 ± 8.5*	74.7 ± 4.9*	65.0 ± 4.7

Example 5

A typical tablet has the composition:

Pentaerythrityl tetranitrate	ISIS PHARMA	20 mg
Lactose	DAB 10	137 mg
Potato starch	DAB 10	80 mg
Gelatin	DAB 10	3 mg
Talc	DAB 10	22 mg
Magnesium stearate	DAB 10	5 mg
Silica, highly disperse	DAB 10	6 mg
		273 mg

Example 6

A tablet containing 20 mg of pentaerythrityl trinitrate (PETriN) has the composition:

	PETriN		20 mg
	Lactose	DAB 10	137 mg
	Potato starch	DAB 10	80 mg
	Gelatin	DAB 10	3 mg
	Talc	DAB 10	22 mg
	Magnesium stearate	DAB 10	5 mg
	Silica, highly disperse	DAB 10	6 mg
			273 mg

Example 7

A tablet containing 20 mg of pentaerythrityl dinitrate (PEDN) has the composition:

	PEDN		20 mg
	Lactose	DAB 10	137 mg
	Potato starch	DAB 10	80 mg
	Gelatin	DAB 10	3 mg
	Talc	DAB 10	22 mg
	Magnesium stearate	DAB 10	5 mg
	Silica, highly disperse	DAB 10	6 mg
			273 mg

Example 8

A tablet containing 20 mg of erythrityl tetranitrate (ETN) has the composition:

	ETN		20 mg
5	Lactose	DAB 10	137 mg
	Potato starch	DAB 10	80 mg
	Gelatin	DAB 10	3 mg
	Talc	DAB 10	22 mg
	Magnesium stearate	DAB 10	5 mg
10	Silica, highly disperse	DAB 10	6 mg
			273 mg

Example 9

A tablet containing 20 mg of isosorbide mononitrate (ISMN) has the composition:

	ISMN		20 mg
15	Lactose	DAB 10	137 mg
	Potato starch	DAB 10	80 mg
	Gelatin	DAB 10	3 mg
	Talc	DAB 10	22 mg
	Magnesium stearate	DAB 10	5 mg
20	Silica, highly disperse	DAB 10	6 mg
			273 mg

Example 10

A tablet containing 20 mg of isosorbide tetranitrate (ISDN) has the composition:

	ISDN		20 mg
5	Lactose	DAB 10	137 mg
	Potato starch	DAB 10	80 mg
	Gelatin	DAB 10	3 mg
	Talc	DAB 10	22 mg
	Magnesium stearate	DAB 10	5 mg
10	Silica, highly disperse	DAB 10	6 mg
			273 mg

Example 11

A tablet containing 40 mg of pentaerythrityl tetranitrate (PETN) and 40 mg of propranolo [sic] hydrochloride has the composition:

15	PETN	40 mg
	Propranolol hydrochloride	40 mg
	Lactose	224 mg
	Potato starch	80 mg
	Gelatin	3 mg
20	Talc	22 mg
	Magnesium stearate	5 mg
	Silica, highly disperse	6 mg
		420 mg

Plus 5 pages of figures

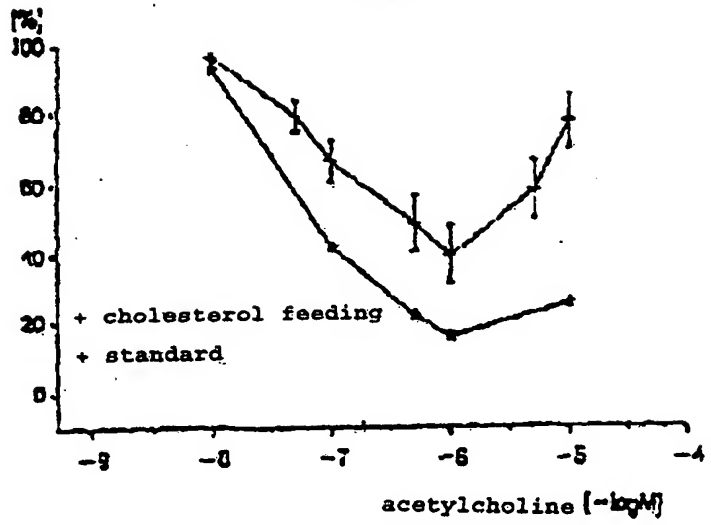

Figure 1:

Development of endothelial dysfunction after feeding of white New Zealand rabbits with a cholesterol diet (0.75%; 15 weeks). The vasorelaxing effect of the endothelium-dependent vasodilator acetylcholine, and thus the functioning of the endothelium, is shown, expressed as the percentage of the precontraction which was induced by the vasoconstrictor phenylephrine and still remained at each concentration given.

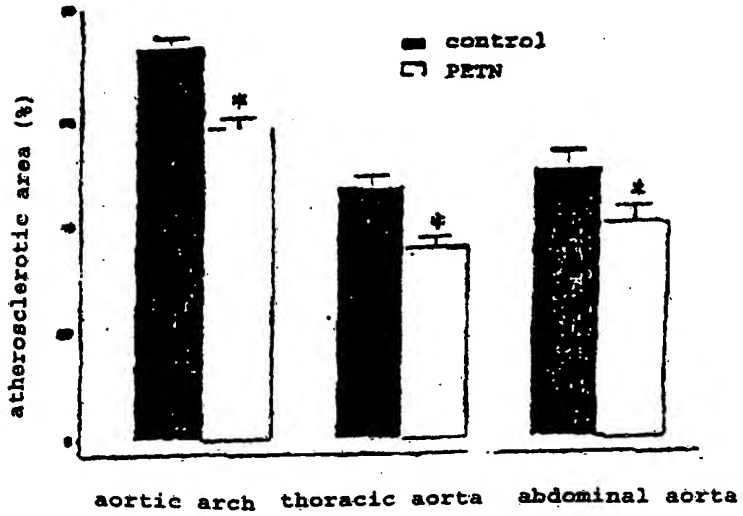

Figure 2:

Extent of atherosclerotic lesions on the luminal surface of various sections of the aorta after feeding with a cholesterol diet (0.75%; 15 weeks) (without control) and concurrent administration of PETN (6 mg/kg/day). The atherosclerotic lesions were stained with Sudan IV, and the percentage of stained area (based on the total area) was determined with the aid of a computer-assisted laser-scanning method. PETN brings about a significant reduction in the formation of atherosclerotic lesions ($p < 0.05$).

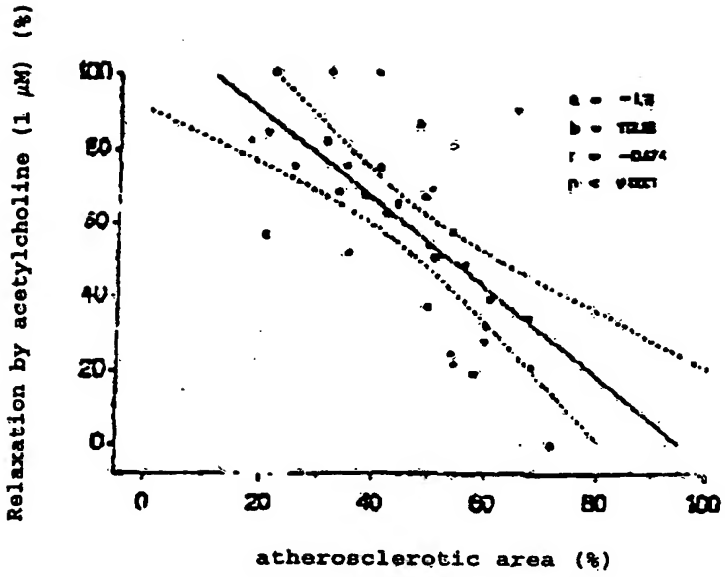

Figure 3:

Relation between the extent of atherosclerotic lesions on the luminal surface of segments of the thoracic aorta after feeding with a cholesterol diet (0.75%; 15 weeks) and the maximum relaxtion, previously determined in the same segment in each case, induced by 1 µM acetylcholine (endothelial function). A larger area of lesions means worse relaxation or endothelial function.

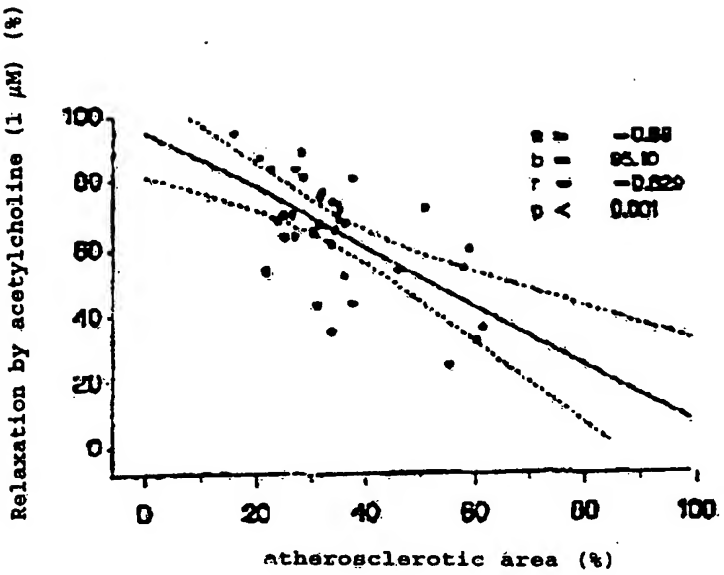

Figure 4:

The same depiction as in Figure 3 after concurrent feeding of cholesterol and PETN.

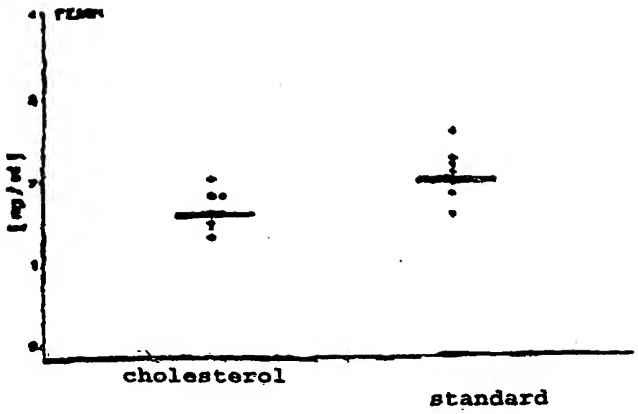

Figure 5:

Plasma levels of pentaerythrityl mononitrate in the plasma of white New Zealand rabbits after withdrawal of feed for 24 h before taking the blood sample preceding the acute test. The standard feed contained in both cases pentaerythrityl tetranitrate (150 mg/kg) and, in the cholesterol group, 0.75% cholesterol in addition. The concentration of pentaerythrityl mononitrate was determined quantitatively by gas chromatography/mass spectroscopy after working up of the plasma samples.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. Method for the prevention, treatment and elimination of endothelial dysfunctions and of disorders associated with these dysfunctions and/or caused thereby, selected from

a) endothelial damage due to hypercholesterolaemia,

b) endothelial damage due to hypoxia,

c) endothelial damage due to mechanical and chemical factors, in particular during and after pharmacological and mechanical reopening of stenosed vessels, for example after percutaneous transluminal angiography [sic] (PTA) and percutaneous transluminal coronary angiography [sic] (PTCA),

d) endothelial damage in the postinfarct phase (endothelial dysfunction associated with reperfusion),

e) endothelial-mediated reocclusion after bypass surgery,

f) disturbances of blood flow in peripheral arteries due to atherosclerotic changes in vessel walls, and atherosclerosis in general,

g) hypertension, including pulmonary and portal hypertension,

i) diabetic micro- and macroangiopathy and

j) heart failure,

by use of compounds which release and/or transfer nitrogen monoxide, with the homocysteinaemia acquired or induced by a genetically related enzyme defect, and the disorders caused thereby, being excepted in each case.

2. Method according to Claim 1, characterized in that the compounds used are chosen from:

a) organic nitrates, in particular glycerol trinitrate (GTN), pentaerythrityl tetranitrate (PETN), isosorbide 5-mononitrate (ISMN), isosorbide dinitrate (ISDN), mannitol hexanitrate, inositol hexanitrate, propatyl nitrate, trolnitrate, nicorandil or SPM 3672, and their pharmacologically suitable derivatives,

b) organic nitrites such as isoamyl nitrite,

c) thionitrites,

d) thionitrates,

e) nitrosothiols such as S-nitroso-N-acetyl-D,L-penicillamine (SNAP),

f) nitrosoproteins, nitrogen monoxide-liberating furoxan derivatives,

g) nitrogen monoxide-liberating sydnone imine derivatives, especially mesocarb, molsidomine or their pharmacologically active metabolites,

h) nitrosyl complex compounds such as iron-nitrosyl compounds, in particular sodium nitroprusside, and

i) nitrogen monoxide (NO) itself.

3. Method according to Claim 1 or 2, characterized in that the compounds are used in combination with other active substances used for the treatment of cardiovascular disorders, in particular with those from the indication groups of ACE inhibitors, antiatherosclerotics, antihypertensives, beta

blockers, cholesterol-lowering agents, diuretics, calcium antagonists, coronary dilators, lipid-lowering agents, peripheral vasodilators or platelet aggregation inhibitors.

Dated this 1 May 1997

ISIS PHARMA GMBH
By their Patent Attorneys
GRIFFITH HACK
Fellows Institute of Patent Attorneys of Australia

Abstract

The present invention describes the use of compounds which release or transfer nitrogen monoxide, of stimulators of endogenous nitrogen monoxide formation and of stimulators of guanylate cyclase for the prevention, treatment and elimination of endothelial dysfunctions and of disorders associated with these dysfunctions or caused thereby, and the use of said compounds for the production of pharmaceutical products for said areas of application.

INTERNATIONAL SEARCH REPORT

Inter. ional Application No
PCT/DE 95/00421

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/44 A61K31/21 A61K31/195 A61K31/34 A61K31/22
A61K33/08 A61K33/26 A61K38/55 A61K31/535

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CARDIOVASC RES (ENGLAND), JUN 1993, VOL. 27, NO. 6, PAGE(S) 990-6, Yaghi MM et al 'Effects of nisoldipine upon endothelial dysfunction following ischaemic and peroxidative injury in the perfused rat heart.'	1-6,11
Y	see the whole document	12
X	WO,A,92 18002 (BRIGHAM AND WOMEN'S HOSPITAL) 29 October 1992	1-5,7,8, 11
Y	see the whole document	12

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 July 1995	26. 07. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Stierman, B

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INTERNATIONAL SEARCH REPORT

Inten nal Application No
PCT/DE 95/00421

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BR J PHARMACOL (ENGLAND), MAR 1992, VOL. 105, NO. 3, PAGE(S) 557-62, Sobey CG et al 'Impaired endothelium-dependent relaxation of dog coronary arteries after myocardial ischaemia and reperfusion: prevention by amlodipine, propranolol and allopurinol.'	1-6,11
Y	see the whole document	12
X	HYPERTENSION (UNITED STATES), OCT 1991, VOL. 18, NO. 4 SUPPL, PAGE(S) II37-42, Clozel M 'Mechanism of action of angiotensin converting enzyme inhibitors on endothelial function in hypertension.'	1,2,9-11
Y	see the whole document	12
X	J CARDIOVASC PHARMACOL (UNITED STATES), JUL 1993, VOL. 22, NO. 1, PAGE(S) 103-11, Kojda G et al 'Nitric oxide liberating, soluble guanylate cyclase stimulating and vasorelaxing properties of the new nitrate-compound SPM 3672.'	1-5,7,8, 11
Y	see the whole document	12
X	Z KARDIOL (GERMANY), 1991, VOL. 80 SUPPL 5, PAGE(S) 3-6, Schror K 'Endotheliale Faktoren und Thrombozytenfunktion.'	1,2,7,8, 11
Y	see the whole document	12
X	J PHARMACOBIODYN (JAPAN), MAR 1992, VOL. 15, NO. 3, PAGE(S) 113-20, Higo K et al 'Protective effects of benidipine hydrochloride (KW-3049), a calcium antagonist, against experimental arterial calcinosis and endothelial dysfunction in rats.'	1-6,11
Y	see the whole document	12
X	J CARD SURG (UNITED STATES), MAR 1993, VOL. 8, NO. 2 SUPPL, PAGE(S) 325-8, Wallace A 'Do deficiencies of endothelial derived relaxing factor contribute to myocardial stunning?'	1-5,7,8, 11
Y	see the whole document	12
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Y	see the whole document	12

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INTERNATIONAL SEARCH REPORT

Intern nal Applicanon No
PCT/DE 95/00421

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citanon of document, with indicanon, where appropriate, of the relevant passages	Relevant to claim No.
X	J INTERN MED (ENGLAND), APR 1994, VOL. 235, NO. 4, PAGE(S) 317-27, Wennmalm A 'Endothelial nitric oxide and cardiovascular disease.'	1,2,7,8, 11
Y	see the whole document	12
X	PRESSE MED (FRANCE), OCT 16 1986, VOL. 15, NO. 35, PAGE(S) 1747-53, Jaillon P 'Traitement de l'angine de poitrine. Perspectives nouvelles.'	1-8,11
Y	see the whole document	12
X	AM J CARDIOL (UNITED STATES), SEP 24 1992, VOL. 70, NO. 8, PAGE(S) 30B-42B, Abrams J 'Mechanisms of action of the organic nitrates in the treatment of myocardial ischemia.'	1-5,7,8, 11,12
Y	see the whole document	12
X	J CARDIOVASC PHARMACOL (UNITED STATES), 1991, VOL. 18 SUPPL 10 PS36-41, Becker RH et al 'Low-dose felodipine treatment attenuates endothelial dysfunction in rabbits fed an atherogenic diet.'	1-6,11
Y	see the whole document	12
X	EUR J PHARMACOL (NETHERLANDS), JUL 2 1987, VOL. 139, NO. 1, PAGE(S) 19-30, Feelisch M et al 'Correlation between nitric oxide formation during degradation of organic nitrates and activation of guanylate cyclase.' see the whole document	1-5,7,8, 11

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DE95/00421

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-12
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Please see annex

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DE95/00421

Incomplete Search
II. Ambiguities,...etc.

Expressions such as "compounds that release and/or carry nitrogen monoxide", "stimulators of guanylate cyclase", "calcium antagonists", "organic nitrates", "thionitrites", etc., "stimulators of kinin receptors", etc., "inhibitors of the angiotensin-converting enzyme", "anti-atherosclerotics", "beta blockers", etc., do not make sufficently clear what specific compounds are meant.

The search was therefore limited to the substances explicitly indicated in the claims and to the general inventive concept.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern .al Application No
PCT/DE 95/00421

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9218002	29-10-92	US-A-	5385937	31-01-95
		AU-B-	660464	29-06-95
		AU-A-	1799192	17-11-92
		CA-A-	2108152	11-10-92
		EP-A-	0590092	06-04-94
		JP-T-	6509323	20-10-94